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Transformation of Intact Yeast Cells Treated with Alkali Cations

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Intact yeast cells treated with alkali cations took up plasmid DNA. Li^+, Cs^+, Rb^+, K^+, and Na^+ were effective in inducing competence. Conditions for the transformation of *Saccharomyces cerevisiae* D13-1A with plasmid YRp7 were studied in detail with CsCl. The optimum incubation time was 1 h, and the optimum cell concentration was 5×10^7 cells per ml. The optimum concentration of Cs^+ was 1.0 M. Transformation efficiency increased with increasing concentrations of plasmid DNA. Polyethylene glycol was absolutely required. Heat pulse and various polyamines or basic proteins stimulated the uptake of plasmid DNA. Besides circular DNA, linear plasmid DNA was also taken up by Cs^+-treated yeast cells, although the uptake efficiency was considerably reduced. The transformation efficiency with Cs^+ or Li^+ was comparable with that of conventional protoplast methods for a plasmid containing *ars1*, although not for plasmids containing a 2μm origin replication.

Yeast cells have been transformed with protoplasts prepared by lytic enzyme treatment (6). However, the preparation of protoplasts and their subsequent regeneration in solid medium are tedious and time consuming, and sometimes protoplasts have low regeneration efficiency. Therefore, the development of more convenient methods for yeast transformation were sought.

Previously (9), Triton X-100, a nonionic detergent, was shown to alter yeast cell membranes so that various extracellular mononucleotides were incorporated into cells. This detergent had no effect on the viability of yeast cells (8). These observations suggested that yeast cells treated with Triton X-100 or other detergents might take up plasmid DNA like *Escherichia coli* cells treated with $CaCl_2$. Therefore, we studied the uptake of plasmid DNAs by intact yeast cells treated with various agents such as detergents and metal ions. Our results show that alkali metal ions such as Li^+, Na^+, K^+, Cs^+, and Rb^+ are effective for inducing competence in yeast cells. On the other hand, detergents were not as effective in inducing competence. By using intact yeast cells treated with these monovalent cations, we could obtain a transformation efficiency that in some cases was comparable with that of the current protoplast method.

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MATERIALS AND METHODS

Strains. *Saccharomyces cerevisiae* D13-1A (a *his3-532 trp1 gal1*) and D308.3 (α *ade1 trp1 his2 met14 hxk1 hxk2 glk2*), gifts from P. K. Maitra, were used as the recipient strains for plasmids YRp7 and YEp6. *S. cerevisiae* AH-22 (a *leu2-3 leu2-112 his4-519 can1*), a gift from N. Gunge, was used as a recipient strain of plasmid pDB248. *E. coli* C600 (F^- *hsdR*$^+$ *hsdM*$^+$ *recA*$^+$ *thr leu thi lacY supE tonA*) was used for the preparation of plasmids YRp7 and YEp6. *E. coli* C600 (F^- *hsdR hsdM recA*$^+$ *thr leu thi lacY supE tonA*) and *E. coli* TrpC9830 (16), gifts from R. W. Davis, were used for plasmid analysis.

Plasmid DNA. DNA from plasmids YRp7 and YEp6 (13) was prepared from cleared lysates of *E. coli* C600. The cleared lysates were prepared by the method of Meyers et al. (11), and plasmid DNA was purified by ethidium bromide-CsCl equilibrium centrifugation at 36,000 rpm (about $10^4 \times g$) in a Hitachi RP65T rotor for 40 h at 20°C. The final DNA preparation after ethanol precipitation was dissolved in TE buffer (Tris-hydrochloride buffer, pH 8.0, containing 1.0 mM EDTA) and was kept at 4°C with chloroform. Plasmid pDB248 (1) was kindly given by N. Gunge. The replication origin of plasmid YRp7 is in *ars1*, whereas those of plasmids YEp6 and pDB248 are in 2μm DNA.

Cultivation, treatment, and transformation. The yeast cells were aerobically grown on 100 ml of YPD medium (1.0% yeast extract, 2.0% polypeptone, 2.0% glucose, pH 5.3) in a 500-ml Sakaguchi flask at 30°C with reciprocation (140 rpm; stroke, 7.0 cm). At the late log phase (optical density at 610 nm, 4 to 8), the cells were harvested by centrifugation at 1,000 × *g* for

5 min, washed once with TE buffer, and suspended in the same buffer to a final concentration of 2×10^8 cells per ml. A 0.5-ml portion of this cell suspension was transferred to a test tube (1.2 by 10.5 cm), and an equal volume of 0.2 M metal ions was added. After 1 h at 30°C with shaking (140 rpm; stroke, 7.0 cm), 0.1 ml of this cell suspension was transferred to a 1.8-ml Eppendorf tube and incubated statically with the addition of 15 μl of a plasmid DNA solution (670 μg/ml) at 30°C for 30 min. An equal volume of 70% polyethylene glycol (PEG)-4000 dissolved in water and sterilized at 120°C for 15 min was then added and mixed thoroughly on a Vortex mixer. After standing for 1 h at 30°C, the Eppendorf tube was immersed into water at 42°C and incubated for 5 min. The cells were immediately cooled at room temperature, washed twice with water at room temperature, and suspended in 1.0 ml of water.

Selection of yeast transformants. For the selection of Trp^+, Leu^+, or His^+ yeast transformants, 0.1 ml of cell suspension prepared as described above was spread on selection agar containing 0.7% yeast nitrogen base, 2.0% glucose, and 50 μg of amino acid per ml or 40 μg of adenine sulfate per ml (L-histidine for Trp^+ and Leu^+ transformants of strains D13-1A and AH-22, respectively; L-tryptophan for His^+ transformants of strain D13-1A; L-histidine, L-methionine, and adenine sulfate for Trp^+ transformants of strain D308.3). The agar petri plates were incubated at 30°C for 2 to 4 days, and the colonies appearing were counted as yeast transformants. The viable cells in the final cell preparations after metal ion treatment and transformation steps were counted by spreading dilutions of cell suspension on YPD agar medium.

Yeast transformation with protoplasts. Yeast cells of strain D13-1A were transformed by the protoplast procedure of Hinnen et al. (6) with the following modifications. Instead of glusulase, Zymolyase 60000 was used. The yeast cells were treated with 10 μg of Zymolyase 60000 per ml in TE buffer containing 1.0 M sorbitol for 1 h at 30°C. Under these conditions, 99% of cells were converted into protoplasts, and the regeneration efficiency of these protoplasts in agar medium was about 11% (data not shown).

Detection of plasmid DNA. Plasmid DNA in Trp^+ yeast transformants was detected by the following two methods. One was direct analysis by agarose gel electrophoresis of cleared lysates prepared from *S. cerevisiae* D13-1A by the method of Cameron et al. (2) with cells growing logarithmically on selective minimal medium. The second method was analysis with cleared lysates prepared from *E. coli* C600. The *E. coli* C600 cells were prepared as follows. Competent cells of *E. coli* C600 after $CaCl_2$ treatment were incubated with the cleared lysates prepared from Trp^+ yeast transformants, and the transformed *E. coli* C600 cells were selected as tetracycline- or ampicillin-resistant (Tet^r, Amp^r) colonies since plasmid YRp7 carries the genes for both Tet^r and Amp^r. Cleared lysates of *E. coli* C600 were prepared by the method of Nagahari (12).

Electrophoresis in agarose gel. Electrophoresis in agarose gel (slab type; 0.7%) was carried out with the buffer described by Helling et al. (5). After electrophoresis, gels were stained with ethidium bromide, and the bands were photographed under UV light.

Digestion of plasmid DNA. Plasmid YRp7 DNA was digested with restriction endonucleases at 37°C overnight in 0.1 ml of a mixture containing (for *Bam*HI) 10 mM Tris-hydrochloride (pH 7.5), 7.0 mM $MgCl_2$, 150 mM NaCl, 0.2 mM EDTA, 7.0 mM 2-mercaptoethanol, and 0.01% bovine serum albumin; or (for *Sal*I) 10 mM Tris-hydrochloride (pH 8.0), 7.0 mM $MgCl_2$, 100 mM NaCl, 2.0 mM 2-mercaptoethanol, and 0.01% bovine serum albumin.

Chemicals. The chemicals used were all analytical grade reagents. Restriction endonucleases were purchased from Takara Shuzo Co., Ltd., Kyoto, Japan. PEG-4000 (average molecular weight, 3,000 to 3,700) was purchased from Nakarai Chemicals Co., Ltd., Kyoto, Japan. Zymolyase 60000 was obtained from Kirin Brewery Co., Ltd., Tokyo, Japan.

RESULTS

Effects of cations. The effects of various cations on the transformation of yeast cells of strain D13-1A with plasmid YRp7 were studied (Table 1). Among the cations tested, Na^+, K^+, Cs^+, and Rb^+ were effective. No significant decrease in the number of viable cells after treatment with these monovalent cations was observed. Li^+ was the most effective of all cations tested, and transformation efficiency with Li^+ exceeded that obtained by the conventional protoplast method.

Detection of plasmid DNAs in transformants. In agarose gel electrophoresis, the cleared lysates prepared from Trp^+ yeast transformants gave two bands corresponding to those of the

TABLE 1. Effect of cations on transformation[a]

Treatment	Viable cells per ml	Transformants per 10 μg of DNA
None	2.6×10^8	0
Metal (0.1 M)		
NaCl	2.3×10^8	540
KCl	2.1×10^8	280
RbCl	1.8×10^8	440
CsCl	2.3×10^8	590
LiCl	2.2×10^8	2,300
Lithium acetate[b]	2.1×10^8	4,000
$MgCl_2$	2.5×10^8	0
$CaCl_2$	2.0×10^8	5
$SrCl_2$	2.6×10^8	5
$BaCl_2$	2.4×10^8	10
$CoCl_2$	2.1×10^8	0
$MnCl_2$	2.6×10^8	0
$ZnSO_4$	1.3×10^8	0
$CuSO_4$	5.6×10^3	0
$Al_2(SO_4)_3$	1.9×10^8	0
Protoplast method		840

[a] Metal ion treatment and transformation were carried out with various metal ions as described in the text.

[b] Lithium acetate, lithium nitrate, and lithium sulfate yielded approximately the same results.

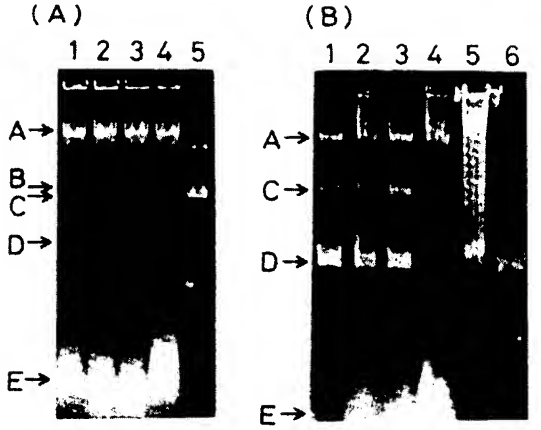

FIG. 1. Plasmid analysis in transformants obtained by CsCl treatment. Cleared lysates of yeast and *E. coli* cells were prepared as described in the text. Electrophoretic conditions were also as described in the text. (A) Electrophoretic patterns of cleared lysates of yeast. Lanes 1 through 3, cleared lysates of Trp^+ yeast transformants; lane 4, cleared lysate of recipient *S. cerevisiae* D13-1A; lane 5, plasmid YRp7 standard. (B) Electrophoretic patterns of cleared lysates of *E. coli* C600. Lanes 1 through 3, cleared lysates of *E. coli* C600 transformed with the DNA in cleared lysates of Trp^+ yeast transformants; lane 4, cleared lysates of recipient *E. coli* C600; lane 5, cleared lysates of *E. coli* TrpC9830 carrying plasmid YRp7; lane 6, plasmid YRp7 standard. Arrows show chromosomal DNA (A), open circular form of 2μm DNA (B), open circular (C) and covalently closed circular (D) forms of plasmid YRp7, and RNA (E).

covalently closed circular and open circular forms of the plasmid YRp7 standard (Fig. 1). On the other hand, cleared lysates prepared from the recipient cells of *S. cerevisiae* D13-1A showed a single band of the open circular form of 2μm DNA, which was also found in all the Trp^+ transformants. Cleared lysates of *E. coli* C600 transformed with DNA in cleared lysates of Trp^+ yeast transformants also showed two bands corresponding to the covalently closed circular and open circular forms of the plasmid YRp7 standard. The positions of these two bands were nearly equal to those of the bands obtained from the cleared lysates of *E. coli* TrpC9830 carrying plasmid YRp7. On the other hand, no transformants of *E. coli* C600 cells incubated with the cleared lysates prepared from the recipient strain *S. cerevisiae* D13-1A were obtained.

Effects of metal ion concentration. The concentrations of Cs^+ and Rb^+ that were best for transformation were 1.0 M and 0.1 M, respectively (Fig. 2). No appreciable loss of viable cells was observed after treatment with these cations.

Effect of metal ion treatment time. Treatment for 1 h was found to be the most effective way of

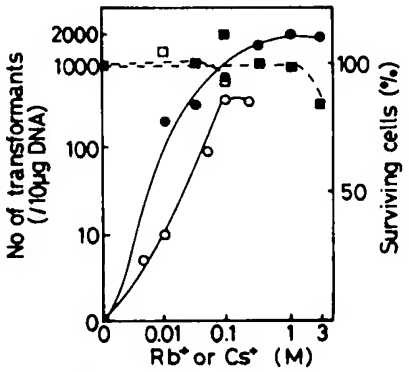

FIG. 2. Effects of Rb^+ and Cs^+ on transformation. Viable cells were transformed and counted as described in the text, except that CsCl (●, ■) and RbCl (○, □) were used at the various concentrations. Symbols: ○, ●, Number of transformants; □, ■, surviving cells.

inducing competence in yeast cells (Fig. 3). Longer incubation with Cs^+ resulted in decreases in both the transformation efficiency and the number of viable cells.

Effect of cell concentration. The optimum cells concentration for treatment with Cs^+ was 5×10^7 cells per ml (Fig. 4), regardless of the plasmid DNA concentration. Transformation efficiency decreased above and below this cell concentration.

Effects of temperature, PEG, and heat pulse. All steps for cation treatment and transformation were carried out at 0 or 30°C. Transformation efficiency was not greatly influenced by temperature (Table 2). However, in the absence of PEG, a marked decrease in transformation efficiency was observed. Heat pulse at 42°C for 5 min also increased the appearance of transformants when the procedures were carried out at 30°C, but not at 0°C.

Effect of plasmid DNA concentration. Trans-

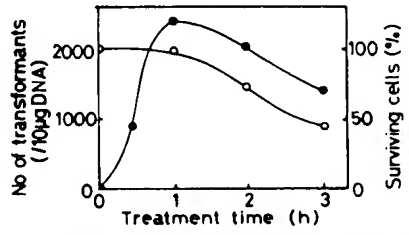

FIG. 3. Effect of treatment time on transformation. Treatment with CsCl was carried out as described in the text, except for the treatment time and CsCl concentration (1.0 M). Other conditions for transformation and determination of viable cells were as described in the text. Symbols: ●, Number of transformants; ○, surviving cells.

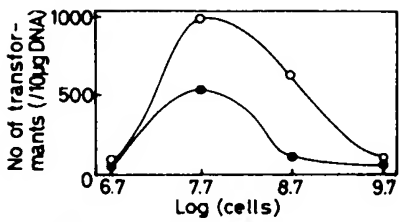

FIG. 4. Effect of cell concentration on transformation. Metal ion treatment was carried out with various concentrations of yeast cells, plasmid YRp7 DNA, and 1.0 M CsCl for 1 h. One-tenth of treated cells were used for transformation. The concentrations of plasmid YRp7 were 25 (○) and 10 (●) μg per 0.1 ml of transformation solution. Other transformation conditions were as described in the text.

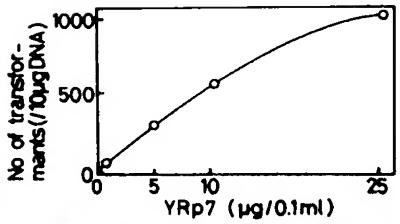

FIG. 5. Effect of DNA concentration on transformation. Cells were treated with 1.0 M Cs^+ for 1 h, and transformation was carried out with plasmid YRp7 at the various concentrations. Other conditions were as described in the text.

formation efficiency increased with increasing concentrations of plasmid DNA (Fig. 5).

Effect of cell age. The effect of cell age on transformation was investigated (Table 3). The highest transformation efficiency was attained by using mid-log-phase cells grown on YPD medium.

Uptake of linearized DNA. In addition to the uptake of the circular plasmid YRp7, the uptake of YRp7 linearized with restriction endonucleases (*Bam*HI and *Sal*I) having a single susceptible site on plasmid YRp7 was investigated (Table 4). Digestion of plasmid YRp7 with these enzymes was carried out at 37°C overnight, and complete digestion was monitored by agarose gel electrophoresis. The linearized plasmid YRp7 was taken up by Cs^+-treated yeast cells, although much less efficiently than circular YRp7. The various basic compounds did not stimulate the uptake of plasmid YRp7 linearized with endonucleases, although the marked increase in uptake efficiency of circular YRp7 was observed in the presence of spermine or histamine. Poly-L-lysine strongly inhibited the uptake of both circular and linearized plasmid DNA. The maintenance of plasmid DNA in Trp^+ yeast transformants obtained with linearized plasmid YRp7 was investigated (Fig. 6). Cleared lysates prepared from Trp^+ yeast transformants showed two bands equivalent to the covalently closed circular and open circular forms of the plasmid YRp7 standard.

Uptake of other plasmid DNA. By using yeast cells treated with lithium acetate, the uptake of plasmids other than YRp7 was investigated (Table 5). For this experiment, two kinds of plasmids were used. One was YEp6 carrying the *his3* yeast structural gene and replicator derived from 2μm DNA (13). The other was plasmid pDB248 harboring the *leu2* yeast structural gene and replicator derived from 2μm DNA. These two plasmids were also taken up by lithium acetate-treated cells, although with efficiencies 500- to 10,000-fold lower than with conventional protoplast transformation.

TABLE 2. Effects of temperature, PEG, and heat pulse on transformation[a]

Temp (°C)	PEG treatment	Heat pulse	Transformants per 10 μg of DNA
0	−	−	0
	−	+	10
	+	−	1,690
	+	+	840
30	−	−	10
	−	+	10
	+	−	1,490
	+	+	2,700

[a] Cells were transformed at 0 or 30°C in the presence (+) or absence (−) of PEG-4000 or heat pulse at 42°C for 5 min. Other transformation conditions were as described in the text.

DISCUSSION

Intact yeast cells treated with alkali cations took up plasmid DNA. The characteristics of this transformation method were as follows. (i) The effective cations were limited to Li^+, Na^+, K^+, Rb^+, and Cs^+ in the alkali metal group of the periodic table (Table 1). On the other hand,

TABLE 3. Effect of growth phase on transformation[a]

Growth phase	Growth[b]	Transformants per 10 μg of DNA
Mid-log	1.6	1,520
Late log	11	640
Stationary	24	745

[a] Cells were cultivated in 100 ml of YPD medium at 30°C with shaking with the addition of 1.0 ml of overnight preculture. Treatment was carried out with 1.0 M CsCl for 1 h. Transformation conditions were as described in the text.

[b] Optical density at 610 nm.

TABLE 4. Transformation of CsCl-treated yeast cells with linearized plasmid YRp7[a]

DNA conformation	Transformants per 10 μg of DNA with addition of:				
	Nothing	Spermine	Putrescine	Histamine	Poly-L-lysine
Circular	650	1,680	700	1,690	230
Linearized with:					
*Sal*I	410	270	420	180	0
*Bam*HI	190	190	160	20	0

[a] Cells were transformed with circular plasmid YRp7 or with YRp7 linearized with *Sal*I or *Bam*HI, in the presence or absence of various basic compounds. Metal treatment was carried out with 1.0 M CsCl for 1 h. Other transformation conditions were as described in the text. The *Sal*I and *Bam*HI endonuclease sites do not occur in the region of the plasmid that is homologous to yeast chromosomal DNA.

TABLE 5. Transformation of lithium acetate-treated *S. cerevisiae* cells with different plasmids[a]

Recipient strain	Plasmid DNA	Transformants per 10 μg of DNA
D13-1A	YRp7	4,540
	YEp6	44
D308.3	YRp7	2,550
AH-22	pDB248	1,250

[a] Cells were transformed as described in the text with 0.1 M lithium acetate and various plasmids and recipient strains of *S. cerevisiae*.

Ca^{2+} and Zn^{2+}, which induce competence in *E. coli* cells (10) and plant protoplasts (14), respectively, were inert with yeast cells. The anionic moiety had either no effect or only a slight one since lithium acetate did show higher efficiency than LiCl. (ii) PEG was required for transformation (Table 2). By analogy with the cases of protoplast fusion (3, 7, 15) and transformation of protoplasts with plasmids (6), the effect of PEG can probably be attributed to changes in membrane charges caused by interactions among negatively charged PEG, monovalent cations, and the yeast cell surfaces. Such changes may induce both conformational changes and aggregation of cells. We are now studying the effects of other polymers, such as positively charged polyethylene imine, to elucidate the intrinsic effect of PEG. (iii) Both circular and linear forms of plasmid DNA were taken up by cells. The uptake of circular plasmids was stimulated in the presence of polyamine or basic protein (Table 4). The uptake efficiency for plasmid DNA linearized with restriction endonucleases was lower than that of circular plasmid DNA. Agarose gel electrophoresis showed that the linearized plasmid DNA taken up was self-ligated in the recipient cells without being integrated into the chromosomal DNA or degraded by nucleases (Fig. 6). In the experiments on the uptake of linear plasmid DNA, various basic compounds were used to protect the cohesive ends generated by restriction endonuclease digestion from the attack of nucleases. However, the addition of such compounds did increase transformation efficiency (Table 4), although an effect of these

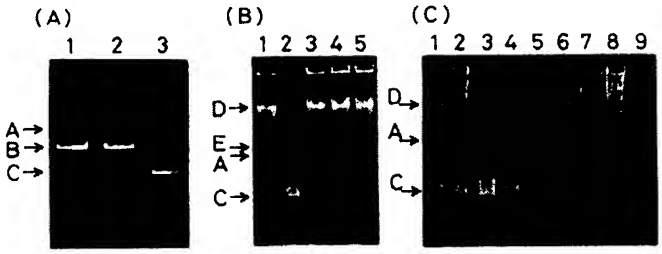

FIG. 6. Plasmid analysis in transformants obtained with linear plasmid DNA. Agarose gel electrophoresis was carried out as described in the text. (A) Electrophoretic patterns of plasmid DNAs used for the transformation. Lane 1, plasmid YRp7 digested with *Bam*HI; lane 2, YRp7 digested with *Sal*I; lane 3, intact YRp7 (circular). (B) Electrophoretic patterns of cleared lysates of Trp^+ yeast transformants. Lane 1, cleared lysates of recipient *S. cerevisiae* D13-1A; lane 2, plasmid YRp7 standard; lanes 3 through 5, cleared lysates of Trp^+ yeast transformants obtained with YRp7 linearized with *Bam*HI (lane 3) or with *Sal*I (lanes 4 and 5). (C) Electrophoretic patterns of cleared lysates of *E. coli* C600. Lanes 1 through 3, cleared lysates of *E. coli* C600 obtained by transformation with DNAs in cleared lysates prepared from Trp^+ yeast cells transformed with plasmid YRp7 linearized with *Bam*HI (lane 1) or *Sal*I (lanes 2 and 3) digestions; lanes 4 through 6, cleared lysates of *E. coli* C600 obtained by incubation with DNAs in cleared lysates of Trp^+ yeast cells transformed with circular plasmid YRp7; lane 7, cleared lysate of *E. coli* C600; lane 8, cleared lysate of *E. coli* TrpC9830 carrying plasmid YRp7; lane 9, plasmid YRp7 standard. Arrows show the open circular (A), linear (B), and covalently closed circular (C) forms of plasmid YRp7, chromosomal DNA (D), and the open circular form of 2μm DNA (E).

compounds was reported on the uptake of linear DNA by tobacco protoplasts (14). On the other hand, a marked stimulatory effect of spermine and histamine on the uptake of circular plasmid DNA was observed. This effect was presumably due to the modification of DNA molecules so as to facilitate entrance through the holes or pores generated by treatment with metal ions and PEG. (iv) Various plasmid DNA species were taken up by cation-treated yeast cells (Table 5). The uptake of various plasmid DNA species was one of the important factors determining the usefulness of our yeast transformation system. Only plasmid YRp7 (*ars1*) was efficiently taken up. Plasmids YEp6 and pDB248 were taken up by *S. cerevisiae* D13-1A and AH-22 with efficiencies that varied from 500- to 10,000-fold lower than with the protoplast method. The poor uptake efficiency of plasmids YEp6 and pDB248 by *S. cerevisiae* cells may be due to incompatibility between 2μm DNA in recipient cells and plasmids carrying the 2μm DNA origin of replication, although this is not found with protoplast transformation.

Various hypotheses to explain the uptake mechanism of polynucleotides into *E. coli* are presented and well summarized by Grinius (4). The chemiosmotic mechanism of DNA transport predicts the obligatory dependence of the DNA transport process on both the electrochemical potential gradient of H^+ ions across the plasma membrane and the rearrangement of phospholipid bilayers, through which polynucleotides or polynucleoproteids can enter the cells. In our transformation system, the uptake mechanism of plasmid DNA remains obscure, but preliminary experiments showed that the uptake of plasmid DNA by cation-treated yeast cells was also driven by the membrane potential. We are now investigating the uptake mechanism in combination with the effect of basic compounds as mentioned above.

The yeast transformation method developed here has the following advantages over the current protoplast method. (i) It is simple, easy, and time saving. (ii) Transformation efficiency is comparable with that of the protoplast method for YRp7 (*ars1*), although it is much less efficient for plasmids with a 2μm origin of replication. (iii) Replica plating of colonies is possible because no regeneration agar is necessary. (iv) The method is applicable to yeast cells that are resistant or sensitive to lytic enzymes.

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